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2 Title: Salivary cortisol and testosterone responses to
3 high-intensity cycling before and after an 11-day
4 intensified training period

5

6 **Keywords** Exercise · Salivary Testosterone · Salivary Cortisol · Endocrine · Endurance · Stress

DRAFT

1 **Abstract**

2 This study examined salivary cortisol and testosterone responses to two, different
3 high-intensity, ~30-min cycles separated by 2 h rest before and after an 11-day
4 intensified training period. Twelve recreationally active, healthy males completed
5 the study. Saliva samples were collected before, immediately after and 30 min
6 after both bouts with salivary cortisol and testosterone concentrations assessed.
7 Compared with pre-training blunted exercise-induced salivary cortisol,
8 testosterone and cortisol/testosterone responses to both bouts post-training were
9 observed ($p < 0.05$ for all). Comparing pre- with post-training the absolute
10 exercise-induced salivary cortisol, testosterone and cortisol/testosterone decreased
11 from 11.1 to 3.1 and 7.0 to 4.4 nmol·L⁻¹ (cortisol), from 407 to 258 and from 473
12 to 274 pmol·L⁻¹ (testosterone) and from 12 to 4 and 7 to 5 (cortisol/testosterone)
13 for the first and second bouts, respectively ($P < 0.05$). No differences in the pre-
14 and post-training RPE and HR responses during the cycles or times to fatigue
15 were found. ($P > 0.05$). Fatigue and Burnout scores were higher post- compared
16 with pre-training ($P < 0.05$).

17
18 These high-intensity exercise bouts can detect altered hormonal responses
19 following intensified training. This test could assess athlete's current hormonal
20 status, reductions in salivary cortisol and testosterone responses suggestive of
21 increased fatigue.

22

1 **Introduction**

2 A successful training programme involves physical overload and avoids an
3 excessive imbalance between training stress and recovery. To improve physical
4 performance an athlete will often progressively overload the body by intensifying
5 their physical training (by elevating volume, duration and/or intensity of training).
6 This intensification of training can lead to a performance decrement for a limited
7 period but following sufficient recovery (days to weeks) a “*supercompensatory*”
8 effect may occur with the athlete exhibiting an enhanced performance when
9 compared to baseline levels (Halson and Jeukendrup, 2004; Hooper *et al.*, 1993;
10 Meeusen *et al.*, 2006 & 2012; O’Toole 1998). This strategy has been termed
11 “functional overreaching” (Meeusen *et al.*, 2006 & 2012). If this intensified
12 training continues the athlete can move into a state of “non-functional
13 overreaching” that will lead to a reduction in physical performance that may not
14 resume for several weeks or months. Despite the benefits of overreaching it is
15 possible to develop the overtraining syndrome if insufficient recovery occurs
16 (Meeusen *et al.*, 2006 & 2012). Full recovery from this syndrome may take many
17 weeks, months or years (Meeusen *et al.*, 2006 & 2012). Signs of overreaching
18 have been reported to occur within a period as short as 7 days of intensified
19 training with limited recovery (Halson *et al.*, 2002). Therefore, identifying a
20 reliable biological marker to monitor training stress would be beneficial to
21 highlight the incidence of overreaching and aid in reducing the risk of developing
22 the overtraining syndrome.

23 Resting circulating cortisol and testosterone concentrations have been examined
24 in athletes as possible biological markers of overreaching and the overtraining
25 syndrome (for review see Urhausen, Gabriel & Kindermann, 1995). Cortisol and
26 testosterone taken together highlight a state of stress by indicating the body’s
27 catabolic/anabolic balance respectively. Much of this research has provided
28 contrasting results which is likely due to the variation of training protocols,
29 training status of the participants, measuring methods and controls for diurnal and
30 seasonal variation of hormones used in these studies. So it is difficult to compare
31 the studies that have been completed on this topic. However, currently there is no
32 strong evidence that resting circulating cortisol and testosterone concentrations

33 and the cortisol/testosterone ratio are reliable markers of overreaching/the
34 overtraining syndrome.

35

36 Perhaps instead of examining the resting levels of these hormones during normal
37 training, overreaching and overtraining an examination of the exercise-induced
38 hormonal responses may give a clearer picture of the endocrine alterations that
39 may occur during these training states. Meeusen *et al.* (2004 & 2010) examined
40 whether the exercise-induced responses of cortisol, adrenocorticotrophic hormone
41 (ACTH), prolactin and growth hormone to short duration, high-intensity exercise
42 could distinguish between normally trained and overreached athletes and athletes
43 in a state of non-functional overreaching and the overtraining syndrome. They
44 developed a test protocol consisting of two maximal cycling exercise bouts
45 separated by 4 h resting recovery. A double exercise protocol was used to
46 examine the hormonal responses to a short-duration, high-intensity cycle while
47 also examining the effect of a short duration (4 h) recovery period on the hormone
48 responses. Meeusen *et al.* (2004) reported that the exercise-induced responses of
49 cortisol and ACTH concentrations to the second exercise bout of a double
50 incremental cycle to fatigue protocol decreased by ~118% (cortisol) and ~73%
51 (ACTH) following a 10-day training period consisting of an increased training
52 load compared with before the training period. Athletes were classed as
53 overreached if their performances on a cycle to fatigue bout decreased following
54 the 10-day training camp compared with before. These findings suggest that the
55 responses of cortisol and ACTH concentrations to short duration, high-intensity
56 exercise are blunted following a period of intensified training. In a follow on
57 study Meeusen *et al.* (2010) reported that the responses of ACTH and prolactin to
58 the second maximal exercise bout of the double cycle to fatigue protocol can
59 distinguish between non-functional overreaching and the overtraining syndrome.
60 Athletes in a state of the overtraining syndrome showed little or no exercise-
61 induced increases in both hormones in response to the second maximal exercise
62 bout whereas non-functional overreached athletes showed large exercise-induced
63 increases in both hormones (~300% (prolactin) and ~600% (ACTH) increases
64 from pre-exercise values).

65

66 The conclusions from Meeusen *et al.* (2004 & 2010) are that the endocrine
 67 responses to short-duration, high-intensity exercise will be altered while
 68 overreached or in a state of the overtraining syndrome. In addition these
 69 alterations may be able to distinguish between states of non-functional
 70 overreaching and the overtraining syndrome. These findings are positive
 71 conclusions in the examination of the endocrine alterations in overreaching and
 72 overtraining. However, the duration and physical demand of the double cycle to
 73 fatigue protocol used by Meeusen *et al.* (2004 & 2010) may make this an
 74 impractical tool to be used in overreached athletes. Reducing the physical and
 75 time demand of this testing protocol would provide a more practical tool. Hough
 76 *et al.* (2011) reported that in a normal trained state robust increases in exercise-
 77 induced salivary cortisol and testosterone concentrations occur in response to a
 78 continuous 30-min, high-intensity cycling bout consisting of alternating blocks of
 79 1 min at 55% maximum work rate (\dot{W}_{\max}) and 4 min at 80% \dot{W}_{\max} (55/80).
 80 Robust elevations of these hormones in response to the 55/80 bout when not
 81 overreached or suffering from the overtraining syndrome should make it easier for
 82 any alterations in these hormones to be highlighted. Therefore the aim of this
 83 present study was to examine the responses of salivary cortisol and testosterone to
 84 the 55/80 cycle bout before and after an 11-day intensified training period. During
 85 this intensified training period the volume of training was increased by 143%. The
 86 majority of this increase in training volume consisted of high-intensity endurance
 87 exercise (~75% peak oxygen uptake ($\dot{V}O_{2peak}$)). This duration of the intensified
 88 training period should be sufficient to induce an overreached/overtrained state
 89 (Halsen *et al.*, 2002; Jeukendrup, *et al.*, 1992; Kirwan *et al.* 1988). To measure
 90 the performance levels of the participants a cycle to fatigue at 70% \dot{W}_{\max} (70) (a
 91 cycle until fatigue or 30 min whichever occurs first) will also be completed 2 h
 92 after completion of the 55/80 bout (30 min cycle). In addition salivary hormone
 93 responses to the 70 bout will also be assessed. The hypothesis of this current study
 94 was that the intensified training period would induce overreaching in the
 95 participants in unison with a deterioration of performance levels in the 70 exercise
 96 bout. In addition the cortisol and testosterone responses to the 55/80 and 70 bouts
 97 would be altered comparing pre- with post-training.

1 **Methods**

2 *Participants*

3 Twelve recreationally active, healthy males volunteered to participate in this
4 study. These individuals would not normally be at risk of overreaching and/or the
5 overtraining syndrome and may be more sensitive to the intensified training
6 compared with a group of elite athletes. The participants' anthropometric and
7 physiological characteristics at baseline are shown in Table 1. Each participant
8 visited the laboratory on 13 separate occasions. All study procedures were
9 approved by the Loughborough University Ethical Advisory Committee.
10 Following approval a full written and verbal explanation of this study and possible
11 risks involved was given to each participant. Written informed consent to take
12 part was obtained from each participant before testing began.

13 *******Place Table 1 here*******

14 *Peak Oxygen Uptake ($\dot{V}O_{2peak}$) Assessment*

15 On the first laboratory visit a continuous, incremental $\dot{V}O_{2peak}$ test was completed
16 on a mechanically braked cycle ergometer (Monark Ergonomic 894E, Vansbro,
17 Sweden). The test began at 95 W and the duration of each stage was 3 min. The
18 work rate was increased at the beginning of each stage by 35 W until volitional
19 exhaustion. Expired gas samples were collected for 1 min into Douglas bags
20 during the final minute of each stage and during the final minute of the exercise
21 test. Expired gas was analysed using an O₂/CO₂ analyser (Servomex 1440,
22 Crowborough, UK) along with a dry gas meter (Harvard Apparatus, Edenbridge,
23 UK) for the determination of the rates of oxygen consumption ($\dot{V}O_2$) and carbon
24 dioxide production ($\dot{V}CO_2$). Heart rate (HR) was recorded continuously using
25 short range radio telemetry (Polar F2, Polar Electro Oy, Kempele, Finland). \dot{W}_{max}
26 was determined using the equation; $\dot{W}_{max} = \dot{W}_{final} + (t/T) \cdot \dot{W}_{inc}$ where \dot{W}_{final} is
27 the power output during the final stage completed, t is the amount of time (s)
28 reached in the final uncompleted stage, T is the duration of each stage (180 s), and
29 \dot{W}_{inc} is the work rate increment (35 W). This calculation was taken from

30 Jeukendrup *et al.* (1996). Power outputs equivalent to 55%, 70% and 80% of
31 \dot{W}_{\max} for each participant were calculated and these values were used as the power
32 outputs during the exercise trials. The work rate equivalent to 75% $\dot{V}O_{2peak}$ was
33 interpolated from the relationship between $\dot{V}O_{2peak}$ ($L \cdot min^{-1}$) and work rate (W).
34 This value was used as the work rate during the training days.

35 Main Trials

36 *REST trial*

37 Each participant completed a resting trial (REST) within 10 days before the first
38 exercise trial. For this trial the participant followed the schema as detailed in
39 Figure 1 except there was no exercise completed in this trial.

40 *Exercise trial*

41 All participants completed two exercise trials, once before (within 3 days
42 before)(pre-training) and 24 h after an 11-day training period which consisted of
43 daily 1.5 h cycle bouts at 75% $\dot{V}O_{2peak}$ (post-training). For the exercise trials each
44 participant followed the schema outlined in Figure 1.

45

46 *****Place Figure 1. Here*****

47

48 Each participant came to the laboratory at 11:30. The exercise trials consisted of
49 two continuous cycle bouts: (1) 30 min continuous cycling of alternating blocks of
50 1 min at 55% \dot{W}_{\max} and 4 min at 80% \dot{W}_{\max} (55/80); (2) cycling at 70% \dot{W}_{\max} for
51 30 min or until fatigue, whichever occurred first (70). The inclusion of the 70 bout
52 was twofold, primarily it was to act as a performance measure but it was also
53 added to examine the influence of the recovery period on the hormone response to
54 exercise. It was thought that fatigue times would be close to 30 min. The purpose
55 of stopping the trial at 30 min was to be able to compare the hormone responses to
56 the 70 bout.

57

58 The 55/80 bout began at 12:00 and finished at 12:30. Following a 2 h resting
59 recovery in the laboratory the 70 bout began at 14:30. HR was collected in the

60 final 30 s of each minute and ratings of perceived exertion (RPE) using a 6-20
61 Borg scale were recorded in the final 30 s of each alternating block. A 52-item
62 Recovery-Stress questionnaire was completed at the beginning of each main trial.
63 The Recovery-Stress questionnaire records the frequency of stress and recovery
64 events over a period of three days and nights. Furthermore, it differentiates
65 nonspecific and sport-specific areas of stress and recovery. The questionnaire
66 consists of 19 stress and recovery scales in total (7 general stress; 5 general
67 recovery; 3 sport stress and 4 sport recovery). In the Recovery-Stress
68 questionnaire 52 there are 53 statements which the participants respond to. The
69 participant's response covers the past 3 days/nights and each answer ranges from
70 never (0) to always (6). Unstimulated saliva samples were collected pre-exercise,
71 immediately post-exercise and 30 min post-exercise for both cycling bouts.

72

73 To avoid circadian rhythm and seasonal variation effects on the hormones all
74 main trials and resting trial took place at the same time of day and during the UK
75 summer months of May to August. For each main trial the subjects consumed a
76 standard breakfast 3 h before testing began. Subjects remained fasted until the end
77 of each main trial but drank water *ad libitum* during this time. The subjects
78 abstained from exercise, caffeine and alcohol intake 24 h before each main trial.
79 All subjects were given instructions on measuring, weighing and recording food
80 intake and were asked to complete a food record diary 24 h before each main trial
81 and were instructed to consume a diet as similar as possible 24 h before each main
82 trial. Total energy and macronutrient intake was determined by use of CompEat
83 version 5.8 software (Nutrition Systems, Oxford, UK). Mean energy intake 24 h
84 prior to each trial was 8.6 ± 2.5 MJ with $50 \pm 15\%$ from carbohydrate, $30 \pm 14\%$
85 from fat and $20 \pm 4\%$ from protein. Body mass was measured in shorts and socks
86 before all trials.

87

88 *Training days*

89 Each participant completed an 11-day training period. Training in the laboratory
90 was completed on 9 of the 11 days of the training period. 5 laboratory training
91 sessions were completed on 5 consecutive days and were followed by 2 recovery
92 days. The remaining 4 laboratory training sessions were completed on 4 days
93 consecutively thereafter. The training sessions took place between 07:00 and

16:00. In order for the participant to be fully recovered for the post-training exercise trial the final training day was completed at least 24 h before the start of the post-training exercise trial. Each training day consisted of 1.5 h cycling at 75% $\dot{V}O_{2peak}$. Gas samples, HR and RPE measurements were collected every 10 min for the first 30 min and then every 15 min to ensure the participants were exercising at the appropriate intensity (Figure 2). If appropriate intensity was not achieved the resistance on the ergometer was amended accordingly to achieve an average of 75% $\dot{V}O_{2peak}$ over the 1.5 h cycle.

*****Place Figure 2. Here*****

Training measures outside laboratory

In addition to the daily 1.5 h cycling exercise in the laboratory the participants were free to undertake further training outside the laboratory. The participants were asked to keep the additional training similar to that they would normally complete in a day. The majority of training outside of the laboratory was completed in the 2 recovery days between training day 5 and 6. Training diaries were completed and HR measurements were recorded for every extra session to confirm what exercise was completed outside of the lab. This HR data was also used to calculate training impulse scores to record the intensity of training completed by the participants outside the lab. Training impulse scores are a way to quantify intensity of training by using the duration of training and the fraction of heart rate reserve (HRR) completed during the training bout. Training impulse scores were calculated as detailed in Jobson *et al.* (2009). The equation used was Training impulse = exercise duration X fraction of HR reserve X e (fraction of HR reserve X b), where e is Euler's number 2.718 and b is a constant which is equal to 1.92 in males. Prior to beginning the study each participant reported their normal training activity (duration and mode) over a 7 day period.

120

Salivary handling and analysis

The participants drank water *ad libitum* during the main trials; however, to avoid the possibility of diluting the saliva sample they were not permitted to drink in the 10 min before saliva sampling. Participants were seated throughout and provided

125 an unstimulated saliva sample by passive dribble into a 7 ml sterile vial (Sterilin,
126 UK) with eyes open, head tilted slightly forward and making minimal orofacial
127 movement. Minimum collection time was 2 min for each subject to allow for
128 collection of sufficient sample volume. All saliva samples were immediately
129 divided into aliquots and stored at -20°C until further analysis. The salivary
130 cortisol and testosterone concentrations were determined using commercially
131 available Enzyme Linked Immunosorbent Assay (ELISA) kits (Salimetrics, PA
132 16803, USA). The mean inter-assay coefficients of variation were 3.2% and 2.5%
133 for cortisol and testosterone, respectively. The mean intra-assay coefficients of
134 variation were 3.2 % and 2.6% for cortisol and testosterone, respectively.

135

136 *Statistical analysis*

137 All data in the text and tables are presented as mean values and standard
138 deviations (*s*). Data were checked for normality, homogeneity of variance and
139 sphericity before statistical analysis. If a data set was not normally distributed,
140 logarithmic transformation was performed on the data. If the data remained not
141 normally distributed following logarithmic transformation non-parametric
142 analysis was completed on the data set. RPE scores recorded during the main
143 trials were analysed using non-parametric tests. When the data sets were
144 parametric a two-way (trial x time) repeated measures analysis of variance
145 (ANOVA) was completed. Significant differences were assessed using Student's
146 paired samples t-tests with Holm-Bonferroni adjustments for multiple
147 comparisons. Statistical significance was set at $P < 0.05$.

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34 *Hormonal measurements*

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36 The average $\pm s$ salivary cortisol and testosterone concentrations during the REST
37 trial were 3.5 ± 1.8 nmol.L⁻¹ and 690 ± 202 pmol.L⁻¹, respectively (Figure 3 &
38 Figure 4). *t*-test analysis indicated that salivary cortisol and testosterone
39 concentrations were not different at post-exercise and 30 min post-exercise
40 compared with the pre-exercise values (either Pre 55/80 or Pre 70 where
41 appropriate) ($P > 0.05$ for all).

42

43 Compared with pre-training blunted salivary cortisol and testosterone exercise-
44 induced (55/80 and 70) responses occurred post-training ($P < 0.05$) (Figure 4 &
45 Figure 5).

46

47 *******Place Figure 4. and Figure 5. here*******

48

49 For the 55/80 bout, the post-exercise salivary cortisol peak increase above the pre-
50 exercise level was 11 nmol.L⁻¹ (210%) (pre-training) and 3 nmol.L⁻¹ (44%) (post-
51 training). In response to the 70 bout peak increases of 7 nmol.L⁻¹ (117%) and 4
52 nmol.L⁻¹ (117%) occurred pre- and post-training, respectively.

53

54 For the 55/80 bout, the post-exercise salivary testosterone peak increase above the
55 pre-exercise level was 407 pmol.L⁻¹ (58%) (pre-training) and 258 pmol.L⁻¹ (37%)
56 (post-training). In response to the 70 bout peak increases of 473 pmol.L⁻¹ (83%)
57 and 274 pmol.L⁻¹ (45%) occurred pre- and post-training, respectively.

58

59 Examined as a ratio (cortisol/testosterone), values were also blunted after the 11-
60 day training period compared with before ($P < 0.05$). Increases of 12 (152%) and
61 4 (40%) in response to the 55/80 bout were found before and after the training
62 period, respectively. In response to the 70 bout of exercise 7 (65%) and 5 (67%)
63 increases were found before and after the training period, respectively (Figure 6).

64

65 *******Place Figure 6. here*******

66

1 **Discussion**

2 This present study aimed to determine the salivary cortisol and testosterone
3 responses to high-intensity cycling exercise (55/80 and 70) before and after an
4 intensified training period. More specifically, it set out to establish if the 55/80
5 cycle bout can highlight alterations in the hormonal responses that occur due to an
6 intensified training period. The 55/80 bout has previously been shown to induce
7 robust elevations in salivary cortisol and testosterone concentrations when not in a
8 state of overreaching or the overtraining syndrome (Hough *et al.*, 2011) and it was
9 hypothesized that this bout would be able to highlight alterations in the cortisol
10 and testosterone responses following a period of intensified training. This
11 intensified training intended to overreach the participants. The observations in this
12 current study established that ~30 min, high-intensity cycle bouts (55/80 and 70)
13 are sensitive enough to highlight reductions in the exercise-induced salivary
14 cortisol, testosterone concentrations and cortisol/testosterone ratio responses
15 following an 11-day endurance training period that occurred when compared to
16 pre-training. The magnitude of the changes from pre- to post-training in the peak
17 salivary hormonal responses to the 55/80 and 70 bouts were reductions in the
18 order of 166% (cortisol) and 21% (testosterone) and 112% (cortisol/testosterone)
19 (55/80) and 0% (cortisol) and 38% (testosterone) and an increase of 2% in
20 cortisol/testosterone ratio. In addition the 11-day training period was sufficient to
21 induce psychological fatigue in the participants as highlighted by the increases in
22 the Recovery-Stress questionnaire stress scores over the course of the training
23 period.

24
25 The blunting of the exercise-induced salivary cortisol responses post-training is in
26 agreement with Urhausen *et al.* (1998). They reported blunted exercise-induced
27 ACTH and a trend for lower exercise-induced cortisol responses in athletes
28 suffering from the overtraining syndrome compared with normally trained
29 athletes. This finding was suggested to be due to a suppression of the
30 hypothalamus-pituitary axis causing a reduced ACTH response and consequently
31 a reduction in the cortisol response to exercise. This suggestion seems plausible as
32 Barron *et al.* (1985) reported decreased basal cortisol levels in marathon runners
33 suffering from the overtraining syndrome. This decrease was linked to a
34 dysfunction in the hypothalamus which was highlighted by a reduction in ACTH

35 secretion in response to an insulin-induced hypoglycaemia in the athletes
36 diagnosed with the overtraining syndrome. Also as reported earlier in this current
37 paper Meeusen *et al.* (2004) reported blunted plasma ACTH and cortisol
38 responses to the second of a double cycle to fatigue protocol when comparing
39 overreached athletes with those that are not in a state of overreaching or diagnosed
40 with the overtraining syndrome. Unfortunately we are unable to confirm if any
41 adaptations occurred in the exercise-induced ACTH over the course of this current
42 study. So it can only be speculated that the blunted salivary cortisol response post-
43 training may be due to a dysfunction of the hypothalamus leading to a reduction in
44 ACTH and therefore causing a reduction in the cortisol response.

45

46 Alternatively Wittert *et al.* (1996) suggested that a desensitization of the adrenal
47 gland could be the cause of no changes in resting plasma cortisol concentrations
48 (03:00 – 09:00 serial sampling) that they observed in ultramarathon athletes
49 compared to controls despite higher plasma ACTH concentrations in the athletes
50 compared with controls. The desensitization of the adrenal gland could be a
51 protective mechanism as constant high cortisol levels would be detrimental to the
52 body as it would likely cause high levels of muscle protein degradation. It is
53 unfortunate that this present study did not measure ACTH and cannot confirm if
54 the 11-day training period had an effect on hypothalamic-pituitary function.
55 However, based on the findings of the previous studies it seems likely that the
56 blunted salivary cortisol response to exercise found in this present study is caused
57 by either desensitization of the adrenal glands or by a dysfunction in the
58 hypothalamus or pituitary gland.

59

60 The reduction in the salivary testosterone levels found in this study could be due
61 to an alteration in the synthesis of testosterone and/or secretion in the testes.
62 Hackney *et al.* (2003) reported reduced testosterone synthesis in the testes in
63 endurance trained males compared with age-matched non-active controls.
64 Testosterone production was measured by the infusion of gonadotropin-releasing
65 hormone in a non-active group and trained runner group and found that the trained
66 runner group had a lower testosterone response to the gonadotropin-releasing
67 hormone than the non-active group. In the present study, the increase in endurance
68 training over the 11-day period could have caused a reduction in testicular

69 production rate of testosterone. Furthermore Cumming *et al.* (1983) reported that
70 a dysfunction in testosterone production in males could be linked to an increase in
71 circulating cortisol levels. Acute hypercortisolism was induced in their
72 participants by insulin or hydrocortisone administration and acute increases of
73 cortisol occurred at the same time that a rapid decrease in circulation testosterone
74 concentrations was seen. These authors suggested an inhibitory effect of cortisol
75 on the luteinising hormone receptors on the Leydig cells leading to a reduction in
76 testosterone production and therefore secretion by the testes. The 11-day training
77 period would have exposed all participants to repeated acute cortisol increases. It
78 is possible that the repeated elevations of cortisol levels experienced over the
79 intensified training period had an inhibitory effect on the luteinising hormone
80 receptor expression on the Leydig cells. This would lead to a reduction in the
81 luteinising hormone induced testosterone production and secretion.

82
83 The physiological responses (HR and RPE) to the 55/80 and 70 bouts did not
84 differ pre- to post-training. In addition there was no significant difference in the
85 time to fatigue in the 70 bouts. Hormonal alterations have often been linked to
86 overreaching and the overtraining syndrome (Barron *et al.*, 1985 and Urhausen *et*
87 *al.*, 1995) which are linked to a deterioration of physical performance. Therefore,
88 it was expected that with this alteration in cortisol and testosterone there would be
89 a reduction in physical performance. One of the purposes of the 70 bout was to
90 measure physical performance before and after the intensified training period. It
91 needs to be recognized that the 70 bout did not give an ideal measure of
92 performance as it was a cycle to fatigue or until 30 min whichever was reached
93 first. This was designed like this as it was hypothesized that the cycle to fatigue
94 time would be less than 30-min for most individuals looking at a previous cycle to
95 fatigue protocol used in our lab of similar intensity (Hough *et al.*, 2011). The
96 cycle to fatigue needed to be long enough to induce a response in cortisol (~20
97 min) but not too long to have a large variation, comparing pre- with post-training,
98 in the hormone responses to the cycle to fatigue due to the duration of cycle.
99 Unfortunately, in this current study 10 out of 12 of the participants reached 30
100 min and therefore it is not a true reflection on performance. The purpose of the
101 cycle to fatigue was twofold. Firstly as a performance measure but also to
102 examine the hormonal response to a second high-intensity cycle bout.

103

104 The novel finding of this current study is the establishment that the 55/80 exercise
105 protocol is sensitive enough to highlight adaptations in salivary cortisol and
106 testosterone caused by an intensified endurance training period. Unlike Meeusen
107 *et al.* (2004 & 2010) who reported hormonal reductions following an intensified
108 training period to the second exercise bout only of their double exercise protocol,
109 this current study reported hormonal alterations in response to both exercise bouts
110 (55/80 & 70) post-training. Perhaps this contrast in results was due to the fact that
111 the cycle to fatigue used by Meeusen *et al.* (2004) did not induce an increase in
112 cortisol when the participants were not overreached or overtrained (i.e. in
113 response to the 1st cycle to fatigue before their 10-day training camp) therefore
114 making it difficult to highlight any alterations that occurred when overreached or
115 overtrained. The 55/80 protocol has been shown to induce robust elevations in
116 salivary cortisol and testosterone concentrations in a normal trained state (Hough
117 *et al.*, 2011). This makes it easy to highlight the hormonal alterations that
118 occurred after the period of intensified training. It should also be noted that no
119 changes were found in the resting (i.e. pre-exercise) salivary cortisol and
120 testosterone concentrations pre- and post-training. This suggests that the exercise-
121 induced adaptations in the salivary hormones cortisol and testosterone reported in
122 this current study occur prior to changes in basal measures of these salivary
123 hormones. The fact that the resting cortisol values have not altered after the
124 intensified training period does not agree with some of the studies mentioned
125 previously in this discussion (Barron *et al.*, 1985) but does with others (Wittert *et al.*
126 *et al.*, 1996). These contrasting findings can be explained to be due to the different
127 states of training the participants were in during these studies. Wittert *et al.* (1996)
128 examined ultramarathon runners with no symptoms of suffering from
129 overreaching or the overtraining syndrome but the participants in Barron *et al.*
130 (1985) were diagnosed with the overtraining syndrome.

131

132 The blunting of the cortisol and testosterone responses to the 55/80 and 70 bouts
133 following an intensified training period coupled with an increase in stress scores
134 in a Recovery-Stress questionnaire suggests that to measure training stress with
135 different methods (questionnaires, hormone response to a stress test) may be
136 useful in order to reduce the incidence of unplanned overreaching or the

overtraining syndrome. This has been suggested previously by Nederhof *et al.* (2008) who in a small group ($n = 3$) of speed skaters examined their responses to different diagnostic tools for overreaching or the overtraining syndrome (Recovery-Stress questionnaire, profile of mood state; reaction time task; hormonal response to double cycle to fatigue protocol) while in different training states (1) not overreaching or overtraining, 2) diagnosed with non-functional overreaching and 3) recovering from non-functional overreaching). They reported a relationship between alterations in exercise-induced cortisol and ACTH concentrations and Recovery-Stress questionnaire scores. Rietjens *et al.* (2005) also examined if severe fatigue could be diagnosed by a combination of parameters (profile of mood state; resting hormone testing; cognitive reaction test). They suggested both the profile of mood state and reaction time performance were sensitive parameters for the detection of overreaching. These studies and this current study give strength to the suggestion that a multi mode approach to measuring of markers of overreaching and/or the overtraining syndrome may be useful.

153

154 Limitations

The performance measure used in this study (70) needs to be recognized as a limitation. A better performance test such as a time trial or a complete cycle to fatigue would have provided a better indication of the influence the training period had on performance levels in our participants. This study cannot claim to have measured this accurately. In addition the reproducibility of the cortisol and testosterone responses to the 55/80 bout needs to be measured. This will confirm that the hormonal alterations reported in this current study are due to the intensified training period and not just a normal variation in the hormonal response to the exercise. This warrants further investigation. It would also be of interest to examine the hormone response to the high-intensity exercise over a normal training period of similar duration to the intensified training period used in this current study. A $\dot{V}O_{2peak}$ test could also have been useful at the end of the intensified training period to examine if the fitness level of the participants had altered over this period. However, it must be noted that the RPE and HR responses to the exercise bouts did not alter pre- to post-training which would suggest that the fitness level of the participants had not altered.

171

172 In conclusion, the 11-day training period increased the participants' Fatigue and
173 Burnout scores in Recovery-Stress questionnaires. Coupled with this, compared
174 with pre-training, blunted exercise-induced salivary cortisol and testosterone
175 responses to high-intensity, 30-min cycling bouts were found at the end of the 11-
176 day training period. Importantly unlike similar studies completed by Meeusen *et*
177 *al.* (2004 & 2010) post-training altered exercise-induced cortisol and testosterone
178 responses were found to the first of two 30-min cycling bouts completed (55/80).
179 A desensitization of the adrenal glands or a dysfunction in the hypothalamus or
180 pituitary gland are the likely causes for the blunted exercise-induced salivary
181 cortisol response following the 11-day training period. A reduction in testosterone
182 synthesis and/or secretion in the testes is the possible cause for the salivary
183 testosterone response to the high-intensity exercise that was observed post-
184 training. The reduced testosterone production and secretion level might be due to
185 an inhibitory effect of high levels of circulating cortisol on the luteinising
186 hormone receptor expression on the Leydig cells in the testes. This study indicates
187 that the 55/80 cycle bout can highlight the exercise-induced salivary cortisol and
188 testosterone changes that occur due to an intensified training period. This test
189 would be a useful assessment of an athlete's hormonal status as this status may
190 change in response to increased training stress as found in this present study.
191 Regular assessment of the salivary cortisol and testosterone responses to the 55/80
192 bout in unison with other training stress measures, for example Recovery-Stress
193 questionnaires and performance measures, might help to reduce the occurrences of
194 unplanned overreaching or the occurrence of the overtraining syndrome.

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279

Figure captions

280 **Table 1** Participant physical and physiological characteristics (mean values with
281 standard deviations in parentheses).

282 **Table 2** Average HR and RPE responses to the pre- and post-training 55/80 and
283 70 bouts (mean values with standard deviations in parentheses).

284

285 **Figure 1.** Schema for the resting and exercise trials.

286 * Resting trial does not contain exercise bouts

287 **Figure 2.** Schema for each laboratory training session on days 1 to 5 and 8 to 11.

288 **Figure 3.** The Recovery-Stress questionnaire Fatigue and Burnout scores pre- and
289 post-training.

290 Values are means.

291 *- Different than Pre-training ($P < 0.05$).

292 **Figure 4.** Salivary cortisol (nmol.L^{-1}) responses to the 55/80 and 70 cycle bouts in
293 the REST (\circ) pre- (\blacksquare) and post- (Δ) training exercise trials.

294 * - Main time effect vs. Pre 55/80 ($P < 0.01$) ** - Main time effect vs. Pre 70 ($P <$
295 0.01) \dagger - Main effect of trial pre-training greater than post-training ($P < 0.01$).

296 **Figure 5.** Salivary testosterone (pmol.L^{-1}) responses to the 55/80 and 70 cycle
297 bouts in the REST (\circ) pre- (\blacksquare) and post- (Δ) training exercise trials.

298 * - Main time effect vs. Pre 55/80 ($P < 0.05$); ** -Main time effect vs. Pre 70 ($P <$
299 0.05); \dagger - Main effect of trial pre-training greater than post-training ($P < 0.05$)

300 **Figure 6.** Salivary C/T ratio responses to the 55/80 and 70 cycle bouts in the
301 REST (\circ) pre- (\blacksquare) and post- (Δ) training exercise trials.

302 * - Main time effect vs. Pre 55/80 ($P < 0.01$); ** -Main time effect vs. Pre 70 ($P <$
303 0.01); \dagger - Main effect of trial pre-training greater than post-training ($P < 0.05$)

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